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Via Federal Express

Document Processing Center (Mail Code 7407M)
Room 6428
Attention: 8(e) Coordinator
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
1201 Constitution Ave., NW
Washington, D.C. 20460

Dear 8(e) Coordinator:

Trimethyl 1, 2, 4-benzenetricarboxylate
CAS # 2459-10-1

This letter is to inform you of the results of three recently completed *in vitro* mutagenicity studies with the above referenced test substance.

The test substance was evaluated for *in vitro* genotoxicity in the bacterial reverse mutation test (Ames test), the mammalian cell gene mutation assay using L5178Y mouse lymphoma cells (MLA test) , and the *in vitro* mammalian chromosome aberration test using Chinese hamster ovary (CHO) cells. All three tests were conducted in both the absence and presence of an exogenous metabolic activation system (Aroclor-induced rat liver S9), and dimethyl sulfoxide (DMSO) was used as solvent and negative control. The results from all three assays are positive for genotoxicity.

1. The test substance was evaluated in the Ames test using the plate incorporation method with tester strains *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537, and *Escherichia coli* WP2 *uvr*A. The test was conducted in two phases; a toxicity-mutation test and a mutagenicity test. No toxicity or test substance precipitation was observed at any dose level with any tester strain in either the absence or presence of S9 activation in either test.

The dose levels evaluated in the mutagenicity test were 333, 667, 1000, 3333, and 5000 µg/plate for all tester strains. A positive (2.03-fold) mutagenic response was observed for tester strain WP2*uvr*A in the absence of S9 metabolic activation at 5000 µg/plate. A dose related increase in mean number of revertants was observed at ≥1000 µg/plate for tester strain WP2*uvr*A in the absence and presence of S9 activation. No other positive mutagenic responses were observed at any dose level or with any tester strain in either the absence or presence of S9 metabolic activation.

2. The mouse lymphoma assay was used to evaluate the ability of the test substance to induce *in vitro* mammalian cell gene mutations. The test was conducted as two 3-hour exposures with S9 metabolic activation, and as 3- and 24-hour exposures without S9 metabolic activation. Based on an initial toxicity test, and toxicity observed in the mutation test, the dose ranges analyzed in the non-activated test system was 200 to 900 for the 3-hr treatment, and 300 to 700 µg/mL for the 24-hr treatment. The dose range analyzed in the S9 activated test system was 600 to 1500 for experiment 1 and 600 to 1200 µg/mL for experiment 2.

The test substance caused statistically significant increases in the mutant frequency (MF) in both in the absence and presence of S9 metabolic activation. In the non-activated test systems, biologically significant increases were observed after 24-hr exposure at the dose levels of ≥ 500 µg/ml, and in the activated system at dose levels of ≥ 500 µg/ml in Experiment 1, and ≥ 600 µg/ml in Experiment 2.

3. The chromosome aberration test was used to evaluate the clastogenic (chromosome breaking) potential of the test substance. Numerical aberrations were also assessed. The test was conducted as a

4-hour exposure with and without S9 metabolic activation, and a 20-hour exposure without S9 metabolic activation.

A preliminary toxicity assay was conducted at a concentration range from 5 to 2522 µg/mL, with the top dose meeting the guideline recommended limit dose of 10 mM. A visible precipitate was observed in the treatment medium at concentrations ≥1000 µg/mL in the beginning and end of the treatment periods. Both pH and osmolality of the highest test substance concentration in medium were within normal ranges.

Based on the findings from the preliminary toxicity assay, the highest concentrations chosen for the chromosome aberration assay were 1000 µg/mL for the 4-hour non-activated test condition, 2522 µg/mL for the 4-hour activated test condition, and 750 µg/mL for the 20-hour non-activated test condition. A visible precipitate was observed in the treatment medium at concentrations ≥1000 µg/mL in the beginning and end of the treatment periods. Substantial toxicity (greater than a 50% reduction in cell growth relative to the vehicle control) was observed at the highest concentration in each test condition. Selection of doses for microscopic analysis was therefore based on these dose concentration levels as well as precipitation.

Cytogenetic evaluations were conducted at 500, 750, and 1000 µg/mL for the 4-hour activated and non-activated test conditions, and at 100, 250, and 500 µg/mL for the 20-hour non-activated test condition. The percentage of cells with structural aberrations in the 4-hour activated test substance-treated group was significantly increased above that of the vehicle control at 500, 750 and 1000 µg/mL ($p < 0.05$, Fisher's exact test). The percentage of cells with structural aberrations in the 20-hour non-activated test substance-treated group was also significantly increased above that of the vehicle control at 500 µg/mL ($p < 0.05$, Fisher's exact test). These observed changes were outside the historical control range for structural aberrations, were accompanied by a concentration-related increase, and are considered biologically significant. The percentage of cells with numerical aberrations in the 4-hour non-activated test substance-treated group showed a significant trend as compared to the vehicle control at 750 and 1000 µg/mL ($p < 0.05$, Cochran-Armitage trend test). The percentage of cells with numerical aberrations in the 4-hour activated test substance-treated group was also significantly increased above that of the vehicle control at 500, 750 and 1000 µg/mL ($p < 0.05$, Fisher's exact test). These observed changes were outside the historical control range for numerical aberrations, and are considered biologically significant.

The findings described above are being reported in accordance with the guidance given in the EPA TSCA Section 8(e) Reporting Guide (June 1991).

Sincerely,